PREVENTIVE EFFECT OF PRECAUTIONARY LOWERED EXPOSURE OR ADAPTATION OF NANOMATERIAL WORKERS?

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SUMMARY

Objectives: Nanotechnology is a fast-growing field in both science and industry. However, experimental studies brought warning data concerning the negative effect of engineered nanoparticle exposure leading to oxidative stress, inflammation, decreased immune cell viability, and genotoxicity. The consequences of human exposure may appear with decades of latency. Therefore, more data is needed to identify the hazardous effects of nanoparticles. Exposure should be under control and biomarkers of effect are urgently searched.

Methods: Exposures of researchers working with nanocomposites were measured in yearly intervals for 5 years and biomarkers of oxidative stress and/or antioxidant capacity were analysed. Exposure to aerosols with nanoparticles was measured repeatedly using online and offline instruments during both the machining of geopolymer samples with epoxide resin and nanoSiO_2 filler and metal surface welding. The levels of biomarkers of oxidation of lipids, nucleic acids and proteins were analysed in exhaled breath condensate (EBC) of researchers and controls in 2016–2018. In 2019 and 2020, glutathione was measured in plasma to assess their antioxidant status. The trends in both exposure and EBC biomarkers' levels were analysed.

Results: On average, 21 researchers were examined yearly (aged 40 ± 5 years, exposure 14 ± 3 years). After 5 years, the mean mass concentration dropped from 0.921 to 0.563 mg/m^3 and mean total number of particle concentrations from 146,106 to 17,621/cm^3. The majority of biomarkers of oxidation of lipids, proteins and nucleic acids decreased ($p < 0.05$) during repeated measurements from the highest levels being mostly found in 2016. Glutathione in plasma in 2019–2020 was elevated ($p < 0.01$) as compared to controls.

Conclusions: The adaptation of long-term exposed researchers may give a plausible explanation. However, to our meaning, the precautionary principle and higher attention of the employers to the potential risk of nanoparticles by reducing nanoparticles exposure by almost one order of magnitude played the key role.

Key words: engineered nanoparticles, oxidative stress, prevention, adaptation, spirometry

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INTRODUCTION

Studies based on human data concerning the effect of inhalation of engineered nanoparticles (size approximately 1–100 nm) at workplaces are limited despite their enormously fast-growing practical use and a large area of technology and research (1).

Several human studies brought rather worrying results, such as those of Liou et al. (2) and others (3, 4), as confirmed later on by a systemic review of Ghafari et al. (5). Also our studies detected nanoparticles in the body fluids of the workers (6), and found elevations of inflammation and oxidative stress markers (7–10) in agreement with the experimental *in vitro* and *in vivo* studies and data on the deleterious effect on cells and organs. In addition, a decrease in spirometry parameters was found in the workers (11).

This was challenging for companies dealing with nanomaterials, and therefore, many of them did not agree to participate in the studies including the examination of their workers. To extend the scientific data, international multicentre prospective studies were started, based on the voluntary participation of workers (12).

Anyway, these warning data may have attracted the attention of the companies and started introducing preventive measures to decrease occupational exposure.

Our findings in 3 years' exhaled breath condensate (EBC) samples in nanocomposites researchers repeatedly showed a significant elevation of markers of oxidative stress in pre-shift samples with further increase of post-shift samples of EBC, as compared to control subjects ($p < 0.001$) (13). The pre-shift increase after a weekend or several days without work exposure is expected to reflect the chronic exposure to nanoparticles, while the post-shift elevation shows the effect of the recent acute exposure (14).

Recently, on the other hand, we have found elevated reduced glutathione (GSH) in these researchers, which could result from their adaptation to oxidative stress after their long-term exposure. GSH plays an important role in the conjugation of reactive protein cysteine residues to prevent oxidative damage and to detoxify electrophiles by the formation of glutathione disulfide (GSSG). Its measurement serves as an estimation of cellular redox metabolism and an assessment of the antioxidant status of the subjects (15). To find the explanation, we have reviewed our five-year measurements of nanoparticle exposure in the same workshops. In this study, the trends in exposure parameters and pre-shift markers levels during the years 2016, 2017 and 2018 were analysed; in addition, the lung function results were compared.

MATERIALS AND METHODS

Study Design

The study was organized in September in the years 2016–2020, at the beginning of the week after a weekend or holidays, and both pre- and post-exposure samples of EBC were collected (16, 17).

Subjects

Nanocomposite researchers studying thermoplastic or reactoplastic composite materials exhibiting new physical characteristics were examined.

The control subjects were living in the same location. They were working as office employees in the same town but were not exposed to aerosols or dust at their workplace.

All subjects were interviewed by occupational physicians using a detailed questionnaire, including occupational history, the type and length of exposure in years, the length of the usual daily exposure in the workshops, and the latency since the last exposure. The personal history focused on previous diseases, medication, lifestyle habits, and regular physical activity. The physical examination of the subjects was performed, and the body mass index was measured.

Aerosol Exposure in the Workshops

The researchers were working in two workshops. Their work included metals surfaces smelting and welding of metal materials on mild steel (containing Fe, Mg, Si, C, and Si) or alloy (Al, Si, Cu, Zn, Fe, Mn, Mg, Cr, Ni) containing nano-additives in workshop 1, and in workshop 2 machining (grinding with corundum discs and milling) of the finished nanocomposites, i.e., of geopolymer samples and epoxide resin with nanoSiO_2 filler, as described earlier (14).

In the initial year, to trace the highest exposure, the measurement was done at two sites of workshop 1, i.e., during smelting (1a) and welding (1b), in addition to workshop 2, where the machining of the samples was performed (Fig. 1). Only during our study in 2017, all researchers worked in workshop 2. In the following years, measurements were done in both workshops.

The usual exposure of the researchers in the workshops lasted approximately 3 hours, the rest of their working day was spent in their offices. The processes and measurements were described in detail (13–15). Data from five years of repeated measurements of the exposure to nanoparticles using static air monitoring online and offline instruments were provided from our database.

Analysis of Biological Samples

Markers of oxidation of lipids, nucleic acids, and proteins were analysed in the EBC of researchers and controls in 2016–2018. These included markers of oxidation of lipids (malondialdehyde, aldehydes C6-C12, 8-isoProstaglandin F2α), nucleic acids (8-hydroxy-2-deoxyguanosine, 8-hydroxyguanosine, 5-hydroxymethyl uracil – focusing on genotoxic effect); and proteins (o-tyrosine, 3-nitrotyrosine) using liquid chromatography-electrospray ionization-tandem spectrometry (LC-ESI-MS/MS), as described by Syslova et al. in our patients with silicosis and asbestosis (18).

In 2019 and 2020, the antioxidant status was assessed by glutathione using spectrophotometry (15, 19).

Spirometry

Lung function measurements were performed by a SpiroPro, Jaeger, Germany. It included forced vital capacity (FVC), inspiratory vital capacity (VCIN), peak expiratory flow (PEF), and forced expiratory volume in 1 s (FEV1) (20).

Environmental Air Pollution

To exclude the potentially interfering effect of environmental air pollution, the air levels of environmental pollutants measured every 30 min were extracted from the National Hydrometeorological Monitoring System*. The nearest stationary monitoring station was localized about 3 km from the ambulatory room for the examination of the subjects. Five parameters were measured: SO_2, NO_2, O_3, and particulate matter (PM)2.5 and PM10 and compared with recommended limits set by the Air Protection Act No. 201/2012 Coll.

Ethical Considerations

The authors have obtained appropriate institutional review board approval and have followed the principles outlined in the Declaration of Helsinki for all human experimental investigations. In addition, the informed consent has been obtained from all participants involved.

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Statistical Analysis

The statistical calculations were realized using QC Expert software 3.3 (Trilobite, Czech Republic) and MS Excel 365 (Microsoft, USA).

The results were characterized using standard statistical tests (normality, arithmetic mean, standard deviations, confidence intervals, etc.). The normalities of data distributions were tested using statistical moments, the D'Agostino normality test for $N < 100$, and the Kolmogorov-Smirnov normality test. The nanoparticle (number and mass) concentrations in the pertinent years were compared using the one-factor ANOVA tests, calculated using the above-mentioned software. The power trendline of nanoparticle concentrations was calculated using MS Excel. The strength and direction of a power relationship were evaluated by correlation coefficient. The differences in biomarkers in pre-shift (morning) EBC in investigated years were evaluated using one-factor ANOVA, F-tests and t-tests (calculated using the above-mentioned software).

RESULTS

Subjects

The numbers of researchers and controls, their general characteristics, and length of exposure are shown in Table 1. There were no statistically significant differences in the number of participants, age, gender, or length of daily exposure in the individual years; similarly, the difference in the mean length of exposure in years did not reach significance. Also, body mass index, smoking, and alcohol consumption did not differ between the two groups studied in any year (all $p > 0.05$). In addition, no difference was found between exposed and control subjects in the prevalence of dyspnoea, cough, or other respiratory symptoms (all $p > 0.05$).

Exposure

The results of workplace aerosol measurements in the past 5 years are presented in Table 2. The total mass concentrations in the two workshops, the total number of concentrations of particles measured (sized < 10 μm), and the proportion of nanoparticles are shown.

There were 1–3 measurements in the workshops yearly in the past 5 years. All the results of the total number concentrations of particles measured in individual workshops are presented in Figure 1. However, the differences in the measurements did not reach a significance level of 0.05 (based on the results of the one-factor ANOVA tests).

The total particle numbers measured reached the highest levels during machining (workshop 2) in 2016, as shown in Figure 1. Therefore, measurements in this workshop were performed every year. Their comparison and significant decrease in concentration characterized by the power trendline and corresponding correlation coefficient ($r = 0.999$) is presented in Figure 2.

Exhaled Breath Condensate

Oxidative Stress Markers

The results of the pre-shift markers in the years 2016–2018 are presented in Figure 3, where the majority of markers showed

Table 1. *General characteristics of the groups including basic exposure data in the researchers*

Group	Researchers	Controls	Researchers	Controls	Researchers	Controls	Researchers	Controls	Researchers	Controls
Year	2016		2017		2018		2019		2020	
Number (male/female)	20 (15/5)	21(15/6)	20 (13/7)	20 (13/7)	21 (16/5)	18 (12/6)	21 (18/3)	21 (16/5)	22 (19/3)	24 (20/4)
Age (years), mean (SD)	42 (11)	38 (9)	39 (11)	40 (7)	40 (12)	45 (13)	41 (5)	40 (4)	40 (5)	39 (4)
Exposure (years), mean (SD)	18 (10)	0	12 (9)	0	14 (9)	0	14 (4)	0	14 (4)	0
Latency since last exposure (hours), mean (SD)	266 (458)	NA	81 (125)	NA	160 (190)	NA	230 (160)	NA	260 (290)	NA
Usual exposure per day (minutes), mean (SD)	101 (60)	NA	128 (87)	NA	123 (16)	NA	126 (41)	NA	113 (41)	NA

SD – standard deviation; NA – not applicable

Table 2. *Exposure measurements in the years 2016–2020 by monitoring during working operations in workshop 1 and workshop 2*

Category/year	Total mass concentration	Total mass concentration	Total number concentration size <10 µm	Total number concentration size <10 µm	Proportion of nanoparticles	Proportion of nanoparticles
Units	mg/m^3	mg/m^3	No/cm^3	No/cm^3	%	%
	Range	Arithmetic mean	Range	Geometrical mean	Range	Arithmetic mean
2016	0.120–1.840	0.921	48,600–526,000	146,106	40–95	65
2017[a]	0.351	0.351	93,900	93,900	96	96
2018	0.179–0.217	0.198	9,310–29,100	19,205	59–90	74
2019	0.129–1.107	0.572	12,700–19,000	15,534	24–91	58
2020	0.291–1.033	0.563	6,900–45,000	17,621	33–75	54

[a]In 2017, only one measurement was made.

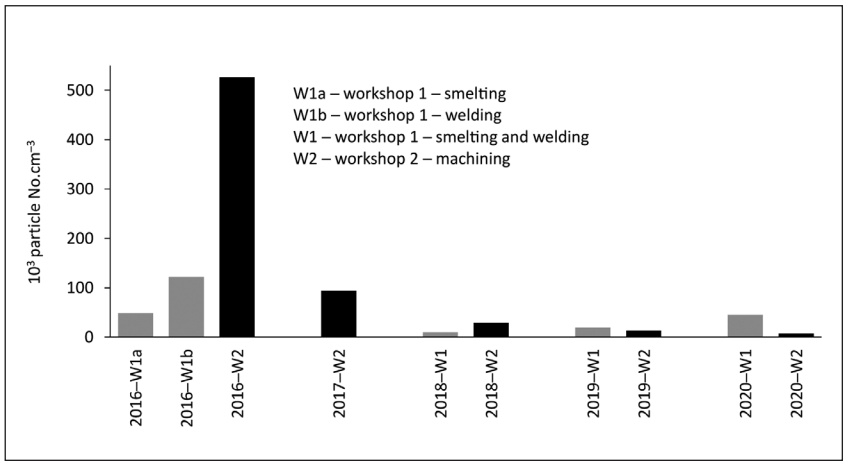

Fig 1. *Measurements of the total number concentrations of particles (size < 10 µm) in the years 2016–2020 in workshop 1 and workshop 2.*

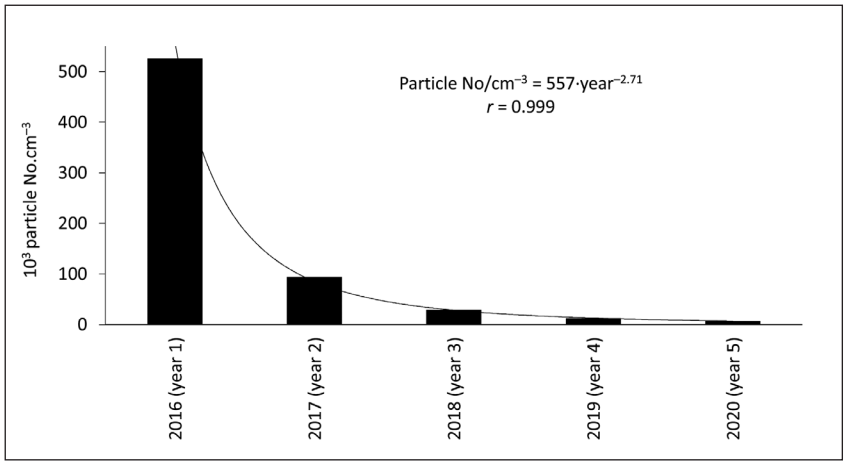

Fig. 2. *Significant decrease in the total number concentrations of particles (size < 10 µm) in workshop 2 during the 5 years' measurement using power trend line model.*

a significant elevation as compared to the controls. The multiple regression analysis confirmed a significant association ($p < 0.05$) between nanocomposite exposure and oxidative stress markers in pre-shift samples of EBC (14). In addition, in 2016, two markers, namely 5-hydroxymethyl uracil, and o-tyrosine were correlated with the length of employment in years in these workshops.

On the other hand, the non-occupational factors, such as age, gender, smoking, consumption of alcohol, and body mass index were not significantly associated with EBC biomarkers.

Glutathione

Also, the biomarker of antioxidant capacity, GSH, was elevated in the plasma of the researchers ($p < 0.01$), as compared to the

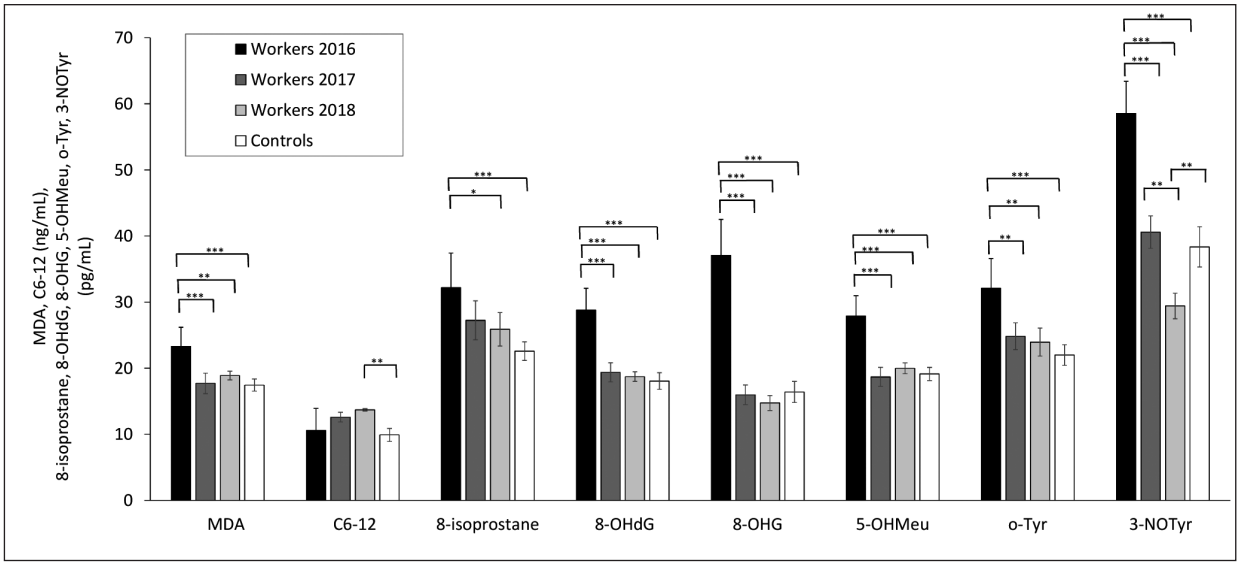

Fig. 3. *Biomarkers in pre-shift (morning) exhaled breath condensate in the years 2016, 2017 and 2018.*

MDA – malondialdehyde; C6-12 – aldehydes C6-C12; 8-isoprostane – 8-isoProstaglandin F2α; 8-OHdG – 8-hydroxy-2-deoxyguanosine; 8-OHG – 8-hydroxyguanosine; 5-OHMeu – 5-hydroxymethyl uracil; o-Tyr – tyrosine, 3-NOTyr – 3-nitrotyrosine

controls (14). The pre-exposure GSH level correlated with the post-exposure level. In all subjects studied, no correlation was found with age, smoking, or consumption of alcohol (Fig. 3).

Spirometry

In 2016 only, two parameters of obstruction changed significantly in the researchers after the shift (pre- vs. post-shift). The %FEV1 decrease from 102.20±13.54 to 99.00±12.03 ($p=0.011$) and the FEV1/FVC ratio from 0.89±0.06 to 0.86±0.06 ($p=0.031$) (21).

In addition, the duration of employment (in years) as a researcher in nanocomposites exposure correlated with a decline in the post-shift FEV1/FVC ratio ($p<0.05$). In the following years 2017–2020, neither post-shit decrease nor significant differences were found in either pre- or post-exposure lung function measurements in the researchers as compared with controls (13).

Environmental Air Pollution

All measured levels of environmental pollutants (SO_2, NO_2, O_3, PM2.5, and PM10) were classified as low or mild for all 5 years and did not exceed the recommended limits. These concentrations did not significantly differ among the four mean levels of measurement.

In all years, no positive correlation of oxidative stress markers with environmental levels was seen.

DISCUSSION

The finding of the elevation of plasma GSH in the researchers in the pre-shift samples in the years 2019 and 2020 could give a plausible explanation and point to the adaptation related to about 14 years of work in this working environment, as discussed by Klusackova et al. (15). Shortly, post-exposure elevation of EBC GSH could be explained as the adaptive response of the respiratory system to the pathological stimulus of nanoparticles based on high level of GSH localized especially in the lining fluid of the lungs (15).

Studies of Rossnerova et al. (22, 23), and Novotna et. al. (24), suggested DNA methylation changes in more than 700 CpG loci across the whole genome associated with chronic inhalation exposure to nanoparticles. Moreover, a comparison of these epigenetic profiles and their changes in exposed researchers and non-exposed controls for four consecutive years revealed highly stable CpG loci in long-term exposed participants and pointed out the process of adaptation and its fixing by epigenetic memory (25).

However, a gradual decrease in the levels of the biomarker collected pre-exposure after several days out of exposure in the years 2016, 2017 and 2018 could reflect a decrease in the chronic exposure of the researchers. The gradually reducing exposure to nanoparticles by almost one order of magnitude could lead to a decrease in the biomarkers of oxidative stress.

Accordingly, in our earlier study in a factory producing TiO_2 with about 80% nanoparticles in the workplace aerosol measured, a decrease of the total mass concentration from 0.40 to 0.22 mg/m^3 in the two following years 2012 and 2013 was seen and lower levels of dust were found in the workshops.

In parallel with the findings in the nanocomposite researchers in this study, also the lung functions of TiO_2 workers showed impairment in the first year (2012), such as for %VCIN and %PEF (both $p<0.01$), especially in the workers with the longest exposure (up to 25 years) in the plant. In addition, titanium concentration in the EBC of the workers negatively correlated with these spirometry parameters. It is in agreement with the recent findings of Squillacioti et al. (26), showing that the cumulative exposure to nanomaterials may worsen pulmonary functions. In the second year of measurement in 2013, this decrease in spirometry in TiO_2 workers has not been seen anymore; also the mean length of exposure of the workers decreased from 10.4 to 8.9 years (11). A recent study by Panizzolo et al. (27) found a relationship between the inhaled dose of nanomaterials and the level of the lung inflammatory biomarkers in the EBC, including

tumour necrosis factor (TNF alpha), which was also elevated in our nanomaterial researchers (21).

Not all biomarkers of oxidative stress reacted in the same way in our studies, because the differences in the oxidative stress biomarkers can be associated with exposure to certain metals, as was recently shown by Sauvain et al. (28).

Unfortunately, the companies' recruitments do not bring large enough numbers of participants. The main problem is the lack of legislation for the regulation of this relatively recent risk (29). On the other hand, there is a greater awareness of emerging risks and a desire to reduce exposure, as seen in this study.

The most severe effects, such as cancers, have not been found in the workers, as they could potentially be expected after several years or decades of latency. Hopefully, such late effects can be decreased to the population level by a substantially lowering exposure, as was observed in several occupational carcinogens. This could be a very positive effect of using the precautionary principle in the practice of occupational medicine.

The limitation of this study is that the oxidative stress biomarkers have not been measured in the last two years, i.e., 2019 and 2020, which was caused by the unavailability of this analysis. However, the samples are stored and can be used for analysis in a laboratory using LC-ESI-MS/MS.

CONCLUSIONS

The decrease of the exposure level was clearly seen in our repeated studies, and even other new studies describe lowering exposures to nanoparticles. This is a piece of positive news related to the effect of the precautionary principle. With such an approach, we may not see similar health damage, as was found in patients with silica or asbestos exposure with elevated markers of oxidative stress in their EBC (30, 31). Anyway, we still cannot exclude this delayed negative effect and further studies in the workers are needed.

To strengthen this preventive effect, it is important to monitor both the workplace aerosol and biomarkers of effect in subjects with engineered nanoparticle exposure. One single EBC biomarker is not sufficient to assess oxidative stress, because they reflect different types of oxidative stress (17, 29). According to our experience, several biomarkers should be measured, including important biomarker of genotoxicity 8-hydroxy-2-deoxyguanosine for the chronic effect, and malondialdehyde for the acute effect. In addition, spirometry should be examined.

Using these precautionary principles, we may prevent potentially serious effects of engineered nanoparticle exposure.

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Conflicts of Interest

None declared

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